SCIENCE 1081147 – Supporting Material

Methods

Proteinase K digestion. Proteins were separated on 12% SDS-PAGE, electrotransferred to PVDF membranes and stained with rabbit antibodies followed by chemiluminescent detection. Proteinase K (Sigma) was added to bacterial cells at 50 μg/ml in RPMI for 30 min at 37ºC. Lysostaphin was added at 20 μg/ml for 30 min at 37ºC.

Heme agarose binding. Purified protein was added to heme-agarose beads (Sigma) at increasing concentrations and incubated at 4ºC for 8 hours in binding buffer (50 mM Tris-HCl pH 7.5, 150 mM NaCl, 0.2% casamino acids). Samples were centrifuged, washed, boiled in SDS, separated on SDS-PAGE and analyzed by immunoblotting. Purified Isd proteins were incubated with human hemoglobin 4ºC for 8 hours in binding buffer and precipitated with glutathione-sepharose beads (Pharmacia). Samples were washed, boiled in sample buffer, separated on SDS-PAGE and bound hemoglobin detected by immunoblotting (Sigma). Quantitations were performed with the Fluorchem program (Alpha-Innotech).

Growth in iron free media. *S. aureus* cultures were grown overnight under iron-starved conditions, washed twice in NRPMI (Chelex treated RPMI) containing 500 μM 2-2' dipyridyl, and incoulated into NRPMI containing 25 μM $ZnCl_2$, 25 μM $MnCl_2$, 1 mM $MgCl_2$, 100 μM $CaCl_2$, 500 μM 2-2' dipyridyl, and grown at 37°C with aeration. 10 μM 98.0% pure heme-iron (hemin) (Fluka) was supplemented as indicated and bacterial growth was monitored in a spectrophotomer (OD_{660}) over time.

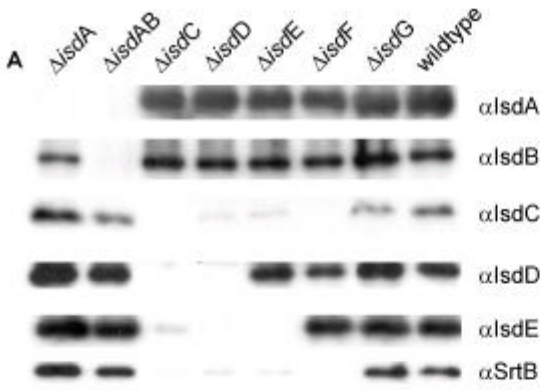
Binding and passage of [^{55}Fe]heme into staphylococci. *S. aureus* strains were grown in Chelex-treated NRPMI containing 25 μM $ZnCl_2$, 25 μM $MnCl_2$, 1 mM $MgCl_2$, 100 μM $CaCl_2$, 500 μM 2-2' dipyridyl, and incubated at 37°C with aeration. When the cultures reached an OD_{660} between 0.4-0.55, the cultures were treated with 1 mM 2-2' dipyridyl for 1 hour. Cells were harvested and suspended in TSM buffer (100 mM Tris-HCl pH 7.0, 500 mM sucrose, 10 mM $MgCl_2$). [^{55}Fe]heme was added to suspensions, and incubated at room temperature for five minutes, at which time ice cold ethanol:acetone [1:1 (vol:vol)] was added to quench iron uptake. Mixtures were incubated on ice for 10 minutes, and subsequently centrifuged at 10,000 ×*g* for 10 minutes at 4°C. The supernatant was aspirated, and bacterial pellets were suspended in 100 μL TSM and subjected to scintillation counting to determine total [^{55}Fe] associated with the bacterial cells. To determine the percent [^{55}Fe] in the protoplast, pellets were suspended in 0.1 M Tris-HCl buffer pH 7.0, and incubated with 100 μg/mL lysostaphin for 10 minutes at 37°C. After digestion, samples were centrifuged at 10,000 ×*g* for 5 minutes, suspended in 0.1M Tris-HCl buffer pH 7.0, and subjected to scintillation counting. Error bars represent standard error of the mean.

Inductively coupled plasma mass spectrometry (ICP-MS). Fe abundances were determined by high-resolution ICP-MS (Thermofinnigan Element1), at medium mass resolving power (R=4000) using $^{56}Fe^{+}$ (exact mass= 55.934940 a.m.u.), where it is spectrally resolved from interfering $^{40}Ar^{16}O^{+}$ (exact mass = 55.957298 a.m.u.). NRPMI solutions were diluted 100-fold, then introduced into the argon plasma with a CETAC-

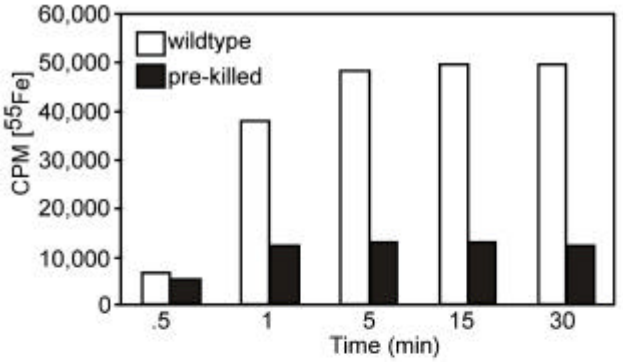
Transgenomic MCN-6000 desolvating nebulizer. Standards were prepared by spiking NRPMI solutions with Spex™ Certiprep high purity Fe solutions. Instrumental sensitivity was $6.3x10^5$ cps/µM Fe (at R=4000). Sample preparations were carried out in a Class-100 clean lab, under HEPA-filtered air, using Seastar™ Nitric acid and 18 MΩ ohm water. Procedural blanks attained were <3 nM.

Fig. S1. Immuno-blot analysis of Isd proteins in *S. aureus* Newman strains carrying mutations in the *isd* locus. Mutations were generated by allelic replacement of coding sequence with the *ermC* gene. Staphylococcal cell extracts were precipitated with TCA and proteins separated on SDS-PAGE. Following electrotransfer to PVDF membrane, the expression of *isd* genes was revealed by immunoblotting with specific rabbit antibodies.

Fig. S2. [^{55}Fe]heme-iron transport across the envelope of *S. aureus* is an active process. Time course of [^{55}Fe]heme-iron co-sedimentation with *S. aureus* cells. White bars represent live wild-type *S. aureus* strain Newman cells, black bars represent heat-killed wild-type *S. aureus*



Mazmanian and Skaar Figure 1 Supplemental



Mazmanian and Skaar Figure 2 Supplemental